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Chaetospermum malipoense sp. nov. from southwest China

Xiao-Ming Tan [1, 2], Chun-Lan Wang[1], Juan Chen[1], & Shun-Xing Guo[1*]

[1] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 151, Malianwa North Road, Haidian District, Beijing 100193, China;*

[2] *Guangxi Botanical Garden of Medicinal Plant, Guangxi Key Laboratory of Medicinal Resources Conservation and Genetic Improvement, Nanning 530023, China*

*Correspondence to: *sxguo1986@163.com*

Abstract —A new *Chaetospermum* species isolated from root of *Coelogyne leucantha* in Yunnan province of southwest China is described as *C malipoense*. It differs from other *Chaetospermum* spp. by the presence of unique conidiomatal setae.

Key words —*Ascomycota,* endophytic fungi, taxonomy

Introduction

Orchidaceae is a diverse family comprising 20,000–35,000 epiphytic and terrestrial species (Cribb et al. 2003; McCormick et al. 2004). Many studies indicate that mycorrhizal fungi play an important role in the stages of seed germination and growth of orchids (Zettler et al. 2004; Yam & Arditti 2009). Additionally, non-mycorrhizal endophytic fungi associated with orchid plants have been found to serve as potential growth promoters and source of bioactivity substances, but studies on these fungi are very limited (Suarez et al 2006).

The genus *Chaetospermum* was established by Saccardo (1892). Ten species and two varieties have been described based on morphological characteristics, e.g., gelatinous conidiomata, conidia with tubular appendages, and holoblastic sympodial conidiogenesis, Molecular analyses have indicated that *Chaetospermum* taxa should be placed in the basidiomycete order, *Sebacinales* (Rungjindamai et al. 2008). Wells & Bandoni (2001) suggested that members of *Efibulobasidium* (*Sebacinales*) represented teleomorphs of *Chaetospermum*. Kirschner & Oberwinkler (2009) also supported this phylogenetic relationship.

During the investigation of endophytic fungi associated with orchid plants in Yunnan province of southwest China, a novel species of the genus *Chaetospermum* was isolated from the root of *Coelogyne leucantha***.** We describe it here as a new species, *Chaetospermum malipoense*.

Materials & methods

Malipo county is located in Wenshan zhuang and Miao minority autonomous prefecture in the southeast of Yunnan province, China, at 22°48′–23°33′N 104°33′–105°18′E. The plant *Coelogyne leucantha* grows epiphytically on the tree trunks in subtropical evergreen forests and flowers in May–June (Flora of China Editorial Board of Chinese Academy of Sciences 1999). In 2010, healthy roots of *C. leucantha* were collected in polyethylene bags, transported to the laboratory and placed in a refrigerator at 4°C. The plant species was identified according to Chen et al. (1999).

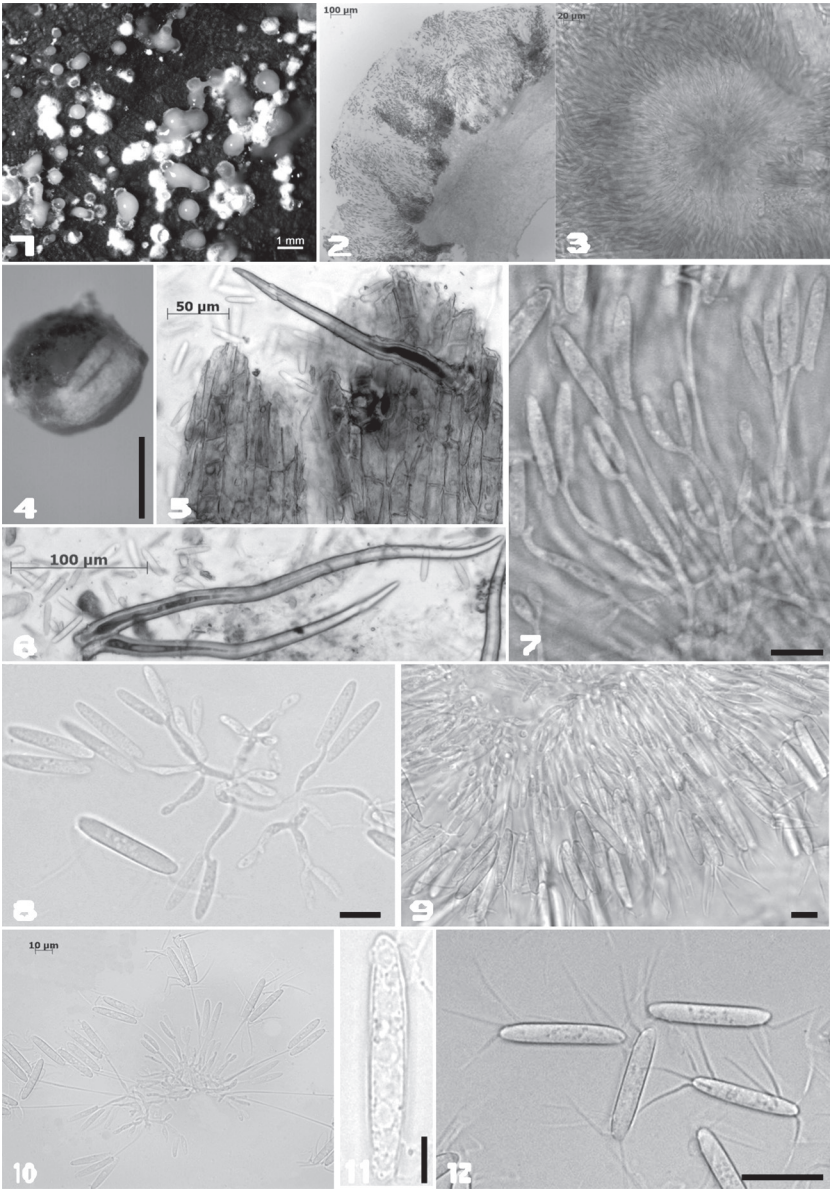
To isolate endophytic fungi, roots of *C. leucantha* washed thoroughly in running tap water were cut into 1 cm segments and surface-sterilized in 70% ethanol for 20 s and 3% $NaClO_2$ for 5 min and rinsed in sterile distilled water 3 times. 3–5 mm segments were separated with the help of a sterile scalpel and were placed on potato dextrose agar (PDA) medium containing 100 µg/ml oxytetracycline and 50 µg/ml streptomycin (Otero et al. 2002). Seven segments were plated per Petri dish, incubated in darkness at 25°C, and checked daily for 4 weeks. Hyphae emerging from segments were transferred to fresh PDA for purity and identification. The pure isolate was morphologically identified and then confirmed by sequence analyses. After extraction of genomic DNA from our isolate, the ITS and 18S rDNA regions were amplified with gene-specific primers (ITS1, ITS4 — White et al. 1990; NS, SL —Inderbitzin et al. 2001) using an iCyclerTM thermal cycler (Mastercycler Gradient). The amplification cycles followed White et al. (1990) and Inderbitzin et al. (2001), respectively. The PCR products were sequenced by Genewiz (Beijing, China) using the same primers as for amplification. Sequences were compared with fungal ITS and 18S rDNA sequences in GenBank using BLAST searches. The fungal isolate was photographed and preserved in the laboratory of Mycology, Biotechnology Center, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences, Beijing; and the culture was accessioned and preserved in China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing. Conidiation structures of *Chaetospermum malipoense* from PDA cultures grown for 20 days at 25°C in darkness were examined, measured, and photographed in a ZEISS Axio Imager A1 (Germany) microscope after mounting in 5% KOH. Measurements were made randomly from 30 cultured specimens and morphology was drawn above tracing paper by free hand and converted into a picture using a scanner.

Taxonomy

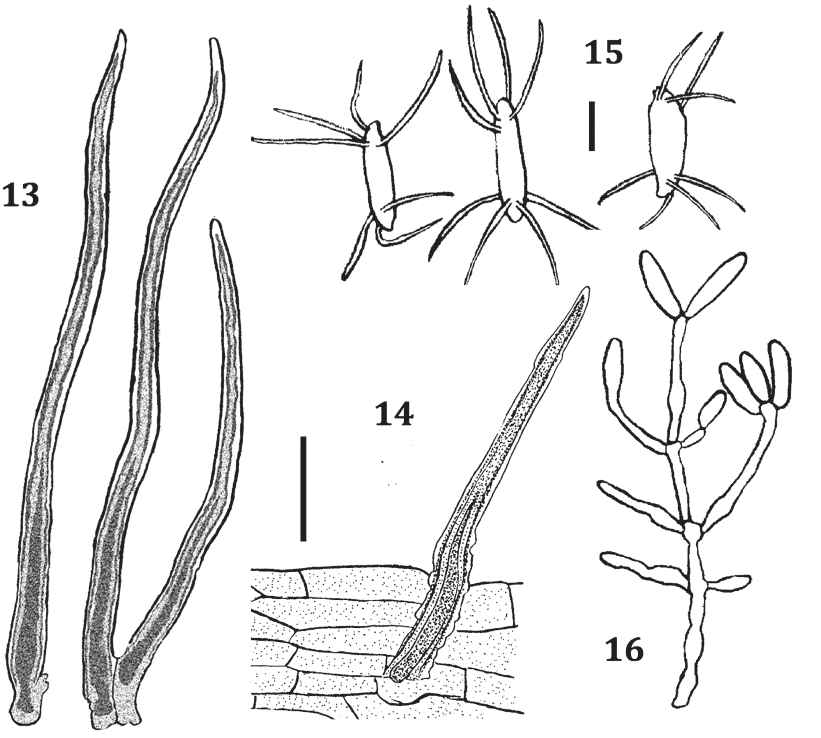
Chaetospermum malipoense X.M. Tan & S.X. Guo, **sp. nov.** Figs 1–16

MycoBank MB 801732

Differs from all other *Chaetospermum* spp. by its aseptate conidiomatal setae and its larger conidia and polar appendages.



Figs 1-12. *Chaetospermum malipoense* (holotype). 1. Habit. 2. Vertical section of conidioma. 3. Conidiophores and conidia. 4. Dehiscent conidiomata. 5. Monothetic conidiomatal setae. 6. Geminate conidiomatal setae. 7–10. Conidiophore branching and conidiogenous cells. 11. Guttulate on the surface of conidia. 12. Conidia with appendages. Scale bars: 2–4 = 250 µm; 5–10 = 10 µm; 11 = 5µm; 12 = 20 µm.



Figs 13-16. *Chaetospermum malipoense* (holotype).
13, 14. Conidiomatal setae. 15. Mature conidia with appendages.
16. Conidiophore branching and conidiogenous cells.
Scale bars: 13, 14 = 50 μm; 15, 16 = 10 μm.

Type: China. Yunnan, Malipo County, in roots of *Coelogyne leucantha* W.W. Sm. (*Orchidaceae*), 17 July 2010, Xiao-ming Tan (**Holotype**, IMPLAD 5880; ex-type culture, CGMCC 6373; GenBank, JQ794486, JQ794487).

Etymology: *malipoense*, referring to the county where the specimen was sampled.

Conidiomata 250–950 μm diameter, opening by a regular split at the base, firstly discrete and globose, ultimately becoming irregular spherical gregarious and confluent, white and gelatinous when moistened. Conidiomatal wall including many layers of non-transparent cells, pseudoparenchymatous at the base, 200–300 μm thick, textura intricata and gelatinous at the upper of conidioma. Setae marginal, 132–362 μm long, 13–23.5 μm wide at the base, light yellow, monothetic to two or three, non-septate at base. Conidiophores arising from the lower half of the conidioma, branched and septate at base,

smooth, firstly fusiform becoming elongate and slender towards the apex with maturity. Conidiogenous cells sympodial, 2–3 in clusters. Conidia mostly cylindrical, smooth, hyaline, (23–)24–32(–37) × (3.5–)4.5–7.5 µm (mean = 27.5 × 5 µm), length/width ratio 5.6:1, abundantly guttulate. appendages subpolar, tubular, unbranched, mostly 3–4 at each end, 14–30 µm long, 1–2 µm wide at the base.

Teleomorph: Unknown.

Comments — We place *C. malipoense* in *Chaetospermum* based on its gelatinous conidiomata, holoblastic sympodial conidiogenesis, and cylindrical non-septate conidia with tubular appendages (Sutton 1980; Nag Raj 1993).

Rajeshkumar et al. (2010) distinguished *C. setosum* from other species by the presence of conidiomatal setae; the 1–2 septa at the base of these setae clearly differ from the aseptate setae of *C. malipoense*. The nearest match for our *C. malipoense* ITS sequence (JQ794486) was an *Efibulobasidium albescens* sequence (AF384860; Wells et al. 2004) with a 95% similarity; however, there are no other *Chaetospermum* ITS sequences in the GenBank database. The nearest match for our *C. malipoense* 18S rDNA sequence (JQ794487) was a *C. camelliae* sequence (EF589729; Rungjindamai et al. 2008) with a 99% similarity. Although *C. camelliae* shares a similar conidial morphology with *C. malipoense,* it can be distinguished by its smaller conidial size (Agnihothrudu 1962).

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